JOURNAL OF CLINICAL MICROBIOLOGY, July 1995, p. 1832–1834 Vol. 33, No. 7
0095-1137/95/$04.00+0
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Rapid Diagnosis of Pulmonary Tuberculosis by Using Roche AMPLICOR *Mycobacterium tuberculosis* PCR Test

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Received 3 February 1995/Returned for modification 3 April 1995/Accepted 21 April 1995

A rapid PCR-based test for the diagnosis of pulmonary tuberculosis, the Roche AMPLICOR *Mycobacterium tuberculosis* test (AMPLICOR MTB), was evaluated. Results from AMPLICOR MTB were compared with culture results and the final clinical diagnosis for each patient. A total of 985 specimens from 372 patients were tested. When AMPLICOR MTB results were compared with resolved results, i.e., a specimen grew *M. tuberculosis* or was obtained from a patient with a clinical diagnosis of tuberculosis, the sensitivity, specificity, positive predictive value, and negative predictive value for the AMPLICOR MTB test were 66.7, 99.6, 91.7, and 97.7%, respectively. These results were comparable to those obtained from culture. Test results were available approximately 6.5 h after specimen receipt in the laboratory. Our data demonstrate that AMPLICOR MTB will provide rapid, valuable information for the diagnosis and control of tuberculosis.

The rapid diagnosis (within 24 h) of infectious diseases, particularly those which represent a public health problem due to their communicability, presents one of the most challenging problems to the clinical microbiologist. The necessity for rapid diagnosis has been underscored by the recent resurgence of tuberculosis in the United States and the increasing frequency of isolation of multidrug-resistant causative strains.

The methodologies routinely used for the laboratory diagnosis of tuberculosis require the growth or isolation of the organisms in a broth or on a solid medium prior to identification. Even with the advent of technological advances such as the BACTEC system (Becton Dickinson Diagnostic Instruments Systems, Cockeysville, Md.) and MB-Chek AFB system (Becton Dickinson Microbiology Systems) and nucleic acid probes, generally, a minimum of 2 weeks is required before a definitive laboratory diagnosis of tuberculosis can be made. Another serious limitation of culture techniques is sensitivity. Although they are considered to be the "gold standard" against which all other methods are measured, their sensitivity leaves much room for improvement (5, 9, 11). Examination of direct smears of clinical specimens for acid-fast bacilli is rapid but lacks sensitivity and specificity.

The most promising diagnostic modality extant to address this problem is PCR. Conceived in 1985 by Mullis (17), PCR permits the exponential amplification of target DNA or RNA molecules. In the clinical microbiology laboratory its application to the detection and identification of fastidious or slowly growing organisms such as mycobacteria, directly in clinical specimens, has the potential to provide a truly rapid laboratory diagnosis of tuberculosis. The major benefits of this rapid diagnostic test are improved patient care, reduced medical costs, and more effective use of isolation rooms.

The use of PCR and other gene amplification assays for the detection of *Mycobacterium tuberculosis* in clinical specimens has been reported (1–4, 6–8, 10, 12–15, 18–20) with promising results. This report summarizes a clinical evaluation of a commercial PCR system, the AMPLICOR *Mycobacterium tuberculosis* test (AMPLICOR MTB; Roche Diagnostic Systems, Somerville, N.J.), and comparison of the PCR assay with culture and microscopic methods for the laboratory and clinical diagnosis of tuberculosis. AMPLICOR MTB is awaiting Food and Drug Administration approval.

MATERIALS AND METHODS

Specimen collection and processing. Specimens were collected from patients suspected to have tuberculosis or who were being monitored for treatment with antituberculosis drugs. A total of 985 sequential specimens from 372 patients were investigated. Specimens were limited to expectorated and induced sputa, bronchoalveolar lavages, and bronchial washings. Specimens which could not be processed on receipt were stored at 2 to 8°C for no longer than 48 h. All were decontaminated and digested by the *N*-acetyl-L-cysteine-NaOH method as described in the *Manual of Clinical Microbiology* (16).

Smear examination. Smears of the digested and decontaminated specimens were stained with the Kinyoun acid-fast stain and examined by standard procedures (16).

Culture. The sediment from a digested and decontaminated specimen was

TABLE 1. Comparison of AMPLICOR MTB results with culture results

Specimen category (no.)	No. of specimens				Sensitivity (%)	Specificity (%)
	Culture positive		Culture negative			
	PCR positive	PCR negative	PCR positive	PCR negative		
All specimens (985)	34	21	14	916	61.8	98.5
Smear negative (949)	21	20	9	899	51.2	99.0
Smear positive (36)	13	1	5[a]	17[b]	92.9	77.3

[a] All five patients had a clinical diagnosis of tuberculosis.
[b] Specimens grew out mycobacteria other than *M. tuberculosis.*

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TABLE 2. Comparison of AMPLICOR MTB results with resolved results[a]

Specimen predictive category (no.)	No. of specimens				Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Resolved positive		Resolved negative					
	PCR positive	PCR negative	PCR positive	PCR negative				
All specimens (985)	44	22	4[b]	915	66.7	99.6	91.7	97.7
Smear negative (949)	26	21	4[b]	898	55.3	99.6	86.7	97.7
Smear positive (36)	18	1	0	17[c]	94.7	100	100	94.7

[a] Culture positive for *M. tuberculosis* or diagnosis of tuberculosis.
[b] Repeat testing in two separate laboratories yielded negative PCR test results.
[c] All specimens were positive for mycobacteria other than *M. tuberculosis.*

suspended to a final volume of 2.5 ml in bovine serum albumin (Becton Dickinson Microbiology Systems), and 0.5 ml of this suspension was used to inoculate an MB-Chek AFB culture bottle (Becton Dickinson Microbiology Systems). The cultures were incubated at 37°C for 8 weeks. They were examined for growth twice a week for the first 2 weeks and then weekly thereafter.

Identification. The identification of isolates belonging to the *M. tuberculosis* complex, *Mycobacterium avium* complex, and *Mycobacterium gordonae* was performed with the Accuprobe tests (Gen-Probe Inc., San Diego, Calif.). All other mycobacteria were identified by standard procedures (16).

Detection of *M. tuberculosis* by AMPLICOR MTB. The AMPLICOR MTB procedure consists of three steps: specimen preparation, amplification, and detection.

Specimens are prepared by addition of 100 μl of concentrated digested-decontaminated specimen to 0.5 ml of wash buffer and then centrifugation at ≥12,500 × *g* for 10 min. The supernatant is aspirated, and 100 μl of lysis reagent is added to the sediment. After vortexing, the suspension is incubated for 45 min at 60°C to complete lysis of the mycobacteria. The lysed material is then neutralized by the addition of 100 μl of neutralization reagent.

AMPLICOR MTB amplifies a 584-bp region of the 16S rRNA gene sequence common to all mycobacteria. Carryover contamination is prevented by incorporation of dUTP in place of dTTP in the amplification reaction and utilization of uracil-*N*-glycosylase (AmpErase) to enzymatically cleave any contaminating amplicon carried over from previous reactions. AmpErase is subsequently inactivated at the temperatures used for thermal cycling. For amplification, 50 μl of neutralized specimen is added to 50 μl of master mix. The tray containing specimens and controls is then placed in a TC-9600 thermal cycler (Perkin-Elmer, Norwalk, Conn.) and amplified according to the following program: hold at 50°C for 2 min; 2 cycles of 98°C for 20 s, 62°C for 20 s, and 72°C for 45 s; 35 cycles of 94°C for 20 s, 62°C for 20 s, and 72°C for 45 s; hold at 72°C for 5 min; and hold at 72°C indefinitely.

Detection of *M. tuberculosis* complex organisms is accomplished by hybridization of the amplified product to a DNA probe specific for organisms of the *M. tuberculosis* complex. Following amplification, 100 μl of denaturation solution is added to all tubes; this is followed by a 10-min room temperature incubation to allow complete denaturation of the double-stranded products. One hundred microliters of hybridization buffer is added to a microwell plate coated with a DNA probe specific for members of the *M. tuberculosis* complex. Twenty-five microliters of denatured amplicon is then added, and hybridization is carried out at 37°C for 90 min. Detection of hybridized duplex is accomplished with an avidin-horseradish peroxidase conjugate–tetramethylbenzidine substrate system. The reaction is stopped by addition of dilute hydrosulfuric acid, and the results are read at 450 nm. A result is considered positive if the absorbance is greater than or equal to 0.35.

Clinical diagnosis of tuberculosis. For the clinical diagnosis of tuberculosis, each patient's chart and chest films were reviewed and pertinent clinical and demographic data and serial X-ray interpretations were recorded.

Statistical methods. Statistical comparisons of sensitivity were calculated by using the McNemar test.

RESULTS AND DISCUSSION

The data in Table 1 demonstrate the correlation between AMPLICOR MTB and culture results. Results from 985 specimens obtained from 372 patients were analyzed. Overall, the sensitivity and specificity of AMPLICOR MTB were 61.8 and 98.5%, respectively. For smear-negative specimens (n = 949) AMPLICOR MTB had a sensitivity of 51.2% and specificity of 99.0%. The sensitivity and specificity for smear-positive specimens (n = 36) were 92.9 and 77.3%, respectively. There was no statistical difference between AMPLICOR MTB and culture results ($P \geq 0.1$).

Table 2 compares PCR results with resolved results, i.e., specimens yielded *M. tuberculosis* on culture or were obtained from a patient with a clinical diagnosis of tuberculosis. This is a more meaningful comparison of data than what is presented in Table 1 because culture, as a test for the diagnosis of tuberculosis, is not 100% sensitive (1–3). For example, in Table 1, five specimens were culture negative, smear positive, and PCR positive. All of these specimens were obtained from patients with a clinical diagnosis of tuberculosis (Table 2). Taking this into account, the specificity of AMPLICOR MTB for smear-positive specimens was 100%, not 77.3%. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for all specimens were 66.7, 99.6, 91.79, and 97.7%, respectively. Four specimens, obtained from patients without a final diagnosis of tuberculosis, were PCR positive and smear and culture negative. Multiple, repeat testing of these specimens in two laboratories yielded consistently negative PCR results. The cause of the false-positive results has not been definitively determined; however, a technical error, possibly occurring during pipetting, is strongly suspected. For smear-negative specimens, the sensitivity, specificity, PPV, and NPV were 55.3, 99.6, 86.7, and 97.7%, respectively. The sensitivity and NPV for smear-positive specimens were 94.7%, and the specificity and PPV were 100%.

Ideally, for the diagnosis of pulmonary tuberculosis at least three successive first-morning specimens should be collected. A comparison of unresolved (clinical diagnosis not considered in data analysis) AMPLICOR MTB and culture results for 372 patients (average of 2.62 specimens per patient) is shown in Table 3. A positive result indicates that at least one specimen for a patient was either PCR or culture positive. Seventeen patients had at least one culture positive for *M. tuberculosis*, and 14 of these patients also had at least one positive AMPLICOR MTB result, resulting in a sensitivity of 82.4%. Seven patients were PCR positive and culture negative. Four of these patients had a single PCR-positive test, most likely due to a technical error (see above). The remaining three patients had

TABLE 3. Comparison of AMPLICOR MTB and culture results for 372 patients[a]

PCR result	No. of patients with culture for *M. tuberculosis*		Sensitivity (%)	Specificity (%)
	Positive	Negative		
Positive	14	7	82.4	98.0
Negative	3	348		

[a] A positive result indicates that one or more specimens for a patient were PCR or culture positive.

TABLE 4. Comparison of AMPLICOR MTB and culture results for patients with and without tuberculosis

Test	Result	Final tuberculosis diagnosis		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive	Negative				
PCR	Positive	17	4[a]	60.7	98.8	81.0	96.8
	Negative	11	333				
Culture	Positive	17	0	60.7	100	100	96.8
	Negative	11	336				

[a] Repeat testing in two separate laboratories yielded negative PCR test results.

a clinical diagnosis of tuberculosis. In this analysis the specificity of AMPLICOR MTB was 98.0%.

A comparison of the capability of PCR and culture to diagnose tuberculosis in the 372 study patients is shown in Table 4. In this analysis, a patient was considered to have tuberculosis if a positive culture was obtained or the clinical presentation was consistent with tuberculosis. Interestingly, both AMPLICOR MTB and culture had identical sensitivities (60.7%). There were three patients with positive AMPLICOR MTB results and negative cultures and a clinical diagnosis of tuberculosis. Also, the three patients had cultures positive for *M. tuberculosis* and negative AMPLICOR MTB results.

The specificity of PCR was 98.8%, and that of culture was, by definition, 100%. PCR would also have been 100% specific but for the probable laboratory error discussed above. This also holds true for the PPV (PCR, 81.0%; culture, 100%). The NPV was 96.8% for both tests.

AMPLICOR MTB compares very favorably to culture in sensitivity, specificity, PPV, and NPV. It has the decided advantage over culture because of its ability to provide a diagnosis of tuberculosis the day of specimen receipt by the laboratory. A reliable, positive result, provided this rapidly, will have a significant impact on patient care and the cost of providing that care. Our data indicate that for patients with pulmonary tuberculosis, AMPLICOR MTB will provide a diagnosis in 6 of every 10 cases the day the test is performed. The test should be used in conjunction with, not in lieu of, culture.

This approach not only will provide rapid, clinically significant results but also may detect cases of tuberculosis missed by culture. At the very least, the test should be performed on all smear-positive specimens and for all specimens from patients considered likely to have pulmonary tuberculosis.

In summary, our data indicate that AMPLICOR MTB provides the clinician, laboratorian, and infection control practitioner with very valuable, rapid, and clinically relevant information for the diagnosis and control of tuberculosis. The test is easy to perform and requires approximately 6.5 h to complete. Four hours and 15 min is incubation or amplification time during which the laboratorian can perform other tasks. A total of 92 specimens can be analyzed during a single test run.

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